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Synopsis of the proposed Research plan

Title of Research Proposal

"PHYTOCHEMICAL ANALYSIS AND EVALUATION OF ANTIMICROBIAL AND ANTI CANCEROUS ACTIVITIES OF *NIGELLA SATIVA, WITHIA SOMNIFERA AND SMILAX ZEYLANICA* PLANTS."

Submitted for the degree of

Doctor of Philosophy in

BOTANY

Under

Faculty of life Science

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Introduction

The confirmatory qualitative phytochemical screening of plant extracts was performed to identify the main classes of compounds like tannins, saponins, flavonoids, alkaloids, phenols, glycosides, steroids, and terpenoids etc. present in the extracts by following standard protocols (Lawal, Lawan, & Apampa, 2019) and (I., E., W., & E. , 2013). Plants are one of the most essential source for all kind of foods and medicine. From ancient time to modern world it is not possible to manufacture the medicine without plant or it's photochemical. They are valuable source of natural active constituents that are used to maintain human health and also used for the treatment of many human diseases (Ammar & Arif, 2019).

Phytochemicals are a powerful group of chemicals that are derived from natural resource, especially with plants origin. They have shown to exhibit chemoprevention and chemotherapeutic effects not only in cell lines and in animal models of Cancer but also some of them are in the clinical trial phase I and II. Despite numerous reports of these phytochemical effects on cancer, an overview of the mechanisms of their action and their effects on various cellular and molecular functions which are important in the inhibition of cancer progression has been lacking (Nasim Faridi , Ahmad Nasimian, & Hamid Heidarzadeh , 2015). Phytochemicals in plants play important roles in their growth and development. They protect plants from harmful agents such as insects and microbes as well as stressful events such as ultraviolet (UV) irradiation and extreme temperatures. They also attract beneficial birds and insects that promote pollination, germination, and seed dispersal.

Phytochemicals provide colours to plants and an array of flavours both pleasant and unpleasant when consumed. They are unique to specific plants and parts of plants, and they usually increase in abundance during stressful events. Phytochemicals also provide health benefits when consumed. They consist of nutrients essential for optimal health (e.g. proteins, carbohydrates, vitamins, and minerals) and other chemicals (e.g., phenolic acids, flavonoids, and other phenolics) with lesser known roles in health promotion or disease prevention. A number of these phytochemicals are recognized as bioactive components in traditional herbal medicines (Kristina , Jessica, & Michael, 2017).

Cancer is a multistage process resulting in an uncontrolled and abrupt division of cells and is one of the leading causes of mortality. The cases reported and the predictions for the near future are unthinkable. Food and Drug Administration data showed that 40% of the approved molecules are natural compounds or inspired by them, from which, 74% are used in anticancer therapy. In fact, natural products are viewed as more biologically friendly, that is less toxic to normal cells (Ana & Diana, 2018) that drugs can be readily administered orally as part of patient's dietary intake (Greenwell & Rahman, 2015).

Plants always have been a great source of drugs, and most of the drugs that are presently available have been originally derived from plants, either directly or indirectly (Tag, Kalita, Dwivedi, Das, & Namsa, 2012). India is recognized as the world's botanical garden because it produces most of the medicinal plants (Seth & Sharma, 2004). Among the list of 21,000 medicinal plants (complied by the WHO) used for therapeutic purposes around the world, 2500 different plant species have been reported in India and 150 species of them are being used economically on a large scale (Modak, Dixit, Londhe, Ghaskadbi, & Devasagayam, 2007).

Among them near about 3000 plants worldwide have been reported to have anticancer properties (Ana & Diana, 2018).

If plant-derived drugs can demonstrate selectivity in research, are non-toxic to normal cell lines and show cytotoxicity in cancer cell lines, these drugs can be lead into clinical trials for further therapeutic development. Plant-derived drugs can fall under four classes of drugs with the following activities; methytransferase inhibitors, DNA damage preventive drugs or antioxidants, histone deacetylases (HDAC) inhibitors and mitotic disruptors (Greenwell & Rahman, 2015).

Although a recent study suggests that nowadays, the traditional medicines are less used, even in populous middle-income countries (Oyinlola , Ngianga-Bakwin, Peter, & Richard, 2016) herbal medicine use is still common in Oncology therapy worldwide (Jorge , Abderrahmane, Thomas, & Lígia, 2017) and (Li, Bin, Yuanxiong, & Hanmin, 2017). The discovery of novel medicinal plants extensively relies on traditional knowledge and the historical literature (E, H, E, T , & B , 2005). Chemically-derived drugs have been developed and other cancer treatments pre-exist. However, current methods such as chemotherapy have their limitations due to their toxic effects on non-targeted tissues furthering human health problems. Therefore, there is a demand for alternative treatments with naturally-derived anticancer agents with plants being the desired source. The secondary metabolites in the plant kingdom have been studied for their potential use as anticancer agents. Collectively they have been shown to possess anticancer activities which include; antioxidant activity; inhibition of cancer cell growth; induction of apoptosis; target specificity; cancer cell cytotoxicity (Greenwell & Rahman, 2015).

The present research study will focus on the antioxidant, antimicrobial and anti cancerous property from three plants; ***Nigella sativa* L.** (belongs to family *Ranunculaceae*), ***Smilax zeylanica* L**. *(Smilacaceae)*, ***Withania somnifera* (L.) Dunal** *(Solanaceae)* they all belongs to different families. The plants will be collect from botanical gardens of Science College, Nanded and from different regions of Maharashtra.

These all are selected on the basis of there medicinal importance, research interest, and phytochemical constituent. The phytochemicals will be isolated from parts of plants to analyze and evaluate essential phytochemicals. After the isolation there antioxidant & antimicrobial activity will be analysed on different pathogens. And on the basis of results, some novel compounds will be selected for analysis and evaluation for anti cancerous properties.
The research will be conduct in Nanded, Maharashtra in Botany research lab at my own research centre Nanded Education Society, Science College, Nanded. The geographical position of Nanded is 19°09'09.9"N latitude and 77°18'58.3"E longitude. And the geographical position of college is 19°10'30.3"N latitude and 77°18'03.6"E longitude.

❖ The selected plants for my study

1. ***Nigella sativa* L.**
2. ***Smilax zeylanica* L.**
3. ***Withania somnifera* (L.) Dunal**

Review of literature

- **(Ana & Diana, 2018)**mention near about 3000 medicinal plants in there study which are anti cancerous in property in through out world.

- **(Ebtesam , et al., 2014)** Perform study on extract and oil of ***Nigella sativa*** and there study conclude that extract and oil significantly reduced the cell viability of human lung cancer cell line, and altered the cellular morphology in a concentration dependent manner. There data also revealed that A-549 cells were more sensitive towards the extract and oil.

- **(Sherma , Agrawal, & Pandey , 2013)** In there study the hydro-methanolic extracts of *Glycyrrhiza glabra* was found to be positive for the presence of so many secondary metabolites. The Zone of Inhibition of ***Glycyrrhiza glabra*** root extract exhibited strong anti bacterial activities for both strain gram (+) and gram (-) bacteria.

- **(Mohammad, Taskim, Sanzida, Md, & Md, 2015)** phytochemical screening and antioxidant activity was tested using several in vitro assays. There findings demonstrate that, the methanolic extracts of ***Smilax zeylanica*** could be considered as a potential source of natural antioxidant agent.

- **(Wafaa, et al., 2018)** study antioxidant and apoptotic induction mechanism of ***Withania somnifera.*** In there study the ashwagandha water extract show antioxidant activity on HepG2 cell, and the study conclude that it is a powerful anti cancer agent. It's Molecular docking revealed that ashwagandha water extract able to bind in the ligand-binding domain of cyclin D1 and inhibit its activity.

- **(Nasim Faridi , Ahmad Nasimian, & Hamid Heidarzadeh , 2015)** suggested about antioxidant properties of plant and they mention about phytochemicals can be a good medicin, as anticancerous drug in there studies.

Objectives of Research

1. To survey the Review of related Literature.
2. Collection and Identification of selected Plant material.
3. Phytochemical analysis (HPTLC) of selected medicinal plants.
4. Investigation of Antioxidant and Antimicrobial properties of plants.
5. To evaluate Anticancer activity of potential plant extract.
6. Perform LC-MS analysis of potential novel compounds for structural information.

Methodology

The methodology for present work is classified in different phases.

Phase 1: Literature survey and collection of plant materials.

In the first phase of study the focus will be on literature review or survey of related literature. The review will be specially focus on the history of plants and there medicinal importance, also on there procedure of extraction. Collection of plant parts from different regions and there accurate identification and maintaining record about the locality of plant materials.

Phase 2 : Plant extraction.

The plant extraction will be carried from collected plant samples by taking help of extraction instruments like soxhlet unit. The phytochemical analysis will be done from different parts of plants.

Phase 3 : Phytochemical evaluation and HPTLC analysis.

The isolated extract will be analyse through HPTLC to identify the presence of secondary metabolites and select them for purpose of study in the next phase.

Phase 4 : Antimicrobial activity from plant parts.

Antimicrobial activity from plant extract of selected 4 plants will be tested against different fungi, bacteria or microbes for their growth inhibition. This will be done by following standard protocols.

Phase 5 : Antioxidant and anti cancerous activity from plant materials.

Antioxidant activity will be study and also, the isolated potential Novel compounds will be selected for further study as an anti cancerous agent.

Phase 6 : LC-MS analysis of selected potential novel compounds.

LC-MS analysis will be carried out to define there molecular structure.

Phase 7 : Thesis writhing and Submission.

Scientific writing of thesis will be done for final submission to University.

Importance of study (society applications)

International status

So many researchers and scientist from abroad and different continent have discover and study on the topic and currently the study is going on.
The study is internationally important in the aspect of cancer treatment.

- In **Egypt** (Wafaa, et al., 2018) perform study on ***Withania somnifera*** and analyse it's antioxidant and anti apoptotic activity on hepatocellular carcinoma cell line which show significant good results from ashwagandha water extract.

- In **Romania** (Raluca, et al., 2020) The Phenolic compounds identified using LC-MS analysis and 13 compounds were identified from ***Nigella sativa*** extract and the compounds are belonging to different classes like phenolic acid, terpenic acid, alkaloids, flavonoids. And in the conclusion of there study, they conclude that the plant it an effective antioxidant agent.

- In **Italy** (Ilaria, Francesca, Camilla, Lorenzo, & Giovanni, 2021) the experiment carried out on root and leaf extract of ***Glycyrrhiza glabra***. There results say that, root extracts contained a two-fold less polyphenol (including flavonoid) content compared to leaf extracts, but with a five-fold lower anti-radical scavenging activity. Only the root extracts provided functional protection in terms of preserving cell proliferation and viability of LPS-treated Caco-2 cells. And the young leaf material offered no anti-inflammatory protection.

- In Colombo, **Sri Lanka**, (N., et al., 2021)The antioxidant, cytotoxic, and anti-cancer potential of aqueous root and rhizome extract (ARRE) of ***Smilax zeylanica*** investigated against human liver carcinoma cells, and the brine shrimp lethality assay showed strong cell killing ability and the extract induced apoptosis of HepG2 cells.

National status

- In **Gujarat,** (Nishant , Badrish , & Nirav , 2009) perform study on antioxidant property of ***Glycyrrhiza glabra*** root extract using in vitro models. And the aqueous and methanolic extract show scavenging activity against nitric oxide, superoxide and hydroxyl radicals. In conclusion, they say that ethanolic extract of *G. glabra* possess considerable antioxidant activity and protective effect against the human lipoprotein oxidative system.

- In **Tamil nadu**, (Arunachalam & Dhanasekar, 2021) perform phytochemical screening by extraction in various solvent. In there results aqueous extract possesses a higher concentration of phenolic compounds and flavonoids, according to quantitative phytochemical study. Conclude that the presence of phytochemicals and free radical scavenging activity in the plant may be responsible for their pharmacological activities and therapeutic effect.

- In **Karnataka,** (Dhanya Shree, Arbin, Saema Noorain, Sahana, & Prashith Kekuda , 2018) there phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, triterpenoids and sterols in both leaf and fruit extracts of ***Smilax zeylanica***, and leaf extract display marked antibacterial activity than fruit extract. In there study Leaf extract caused highest inhibition of test fungi than fruit extract. Susceptibility to extracts was greater in case of *Bipolaris* species than *Aspergillus niger*.
- In **Karnataka**, (Jayanthi, SubbaRao, Divya , K., & Manjula, 2017) evaluate anti-tumor activity of ethanolic extract of ***Glycyrrhiza glabra*** against Ehrlich ascites carcinoma in swiss albino mice, the conclude that the root Extracts of *Gg* showed a significant reduction in % increase in tumor induced body weight of the mice.

Significance of the study

Cancer is one of the most dangerous and world wide spread disease in humans. And as it is one of the fast spreading and easily evolving disease in humans require serious care and precautions. And as increasing worlds population day by day, the cancer variants also getting progress for saviour and multi tasking symptoms hence the demand for medicines for it's treatment increasing through out world. As it's medicines evolving through out the world, no doubt so many laboratories making chemical drugs and other giving priority for the phytochemical drugs, as there easy availability and safe uses for body and many other harmless properties. The humans are searching new drugs within plant to fight with the diseases because of there good results. The plant diversity is so vast on planet earth everyone searching within a plants for novel compounds hence it can be a good time for us to study on a particular plants for there various anti oxidant, anti cancerous activities. So many researchers proved that, the phytochemicals keep us in safe zone because of there harmless characteristic. In other case chemical drugs are may be harmful for the healthy parts of body.

The selected plants for this research show antioxidant, antimicrobial and so far anti cancerous activity, hence I want to explore more about them as I select them on the basis of there phytochemical constituents and literature review so the further study can be done on them to develop an extra importance for them in society and industry.

Formulation and Structure of Study:
Year-wise Plan of work and targets to be achieved

Sr. no.	Year	Activity	Tentative Duration
1)	First Year	• Literature survey and Review of Literature • Collection and accurate identification of plant • Extraction of plant materials in laboratories	12 Months
2)	Second Year	• Phytochemical analysis of plant extract • Antimicrobial activity of plant extract • Antioxidant activity of plant extract	12 Months
3)	Third Year	• Evaluation of anti cancerous activity of plant extract • LC-MS analysis of plants • Data analysis and Thesis writing for submission.	12 Months

- **Presentation of the research work, attending conferences, workshops will be done alongside all the activities as per mentioned in above table.**

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